Pediatric Carbapenem-resistant Enterobacteriaceae in Los Angeles, California, a High-prevalence Region in the United States

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Abstract

Background—Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) are on the rise worldwide but are not well described in pediatric populations. This study characterizes the clinical, phenotypic and genotypic characteristics of CRE infections at a free-standing US children's hospital.

Methods—CRE were defined as any clinical Enterobacteriaceae isolate non-susceptible to either imipenem or meropenem and resistant to ceftriaxone, cefotaxime and ceftazidime determined by routine antimicrobial susceptibility testing. The modified Hodge test was performed to screen for the production of carbapenemase. Clinical data were reviewed, and molecular characterization of phylogenetic and resistance-associated traits was performed.

Results—CRE isolates were recovered from sterile and non-sterile sites in 10 patients, 6 weeks to 24 years of age, between 2011 and 2013. Comorbidities included hematologic, genetic and urologic abnormalities. Two patients had traveled abroad (India, Lebanon) before CRE recovery. Carbapenemase determinants were detected in 5 cases, including KPC-3 in 2 *Klebsiella pneumoniae* (ST258 and ST18) and 1 *Escherichia coli* (ST131), and NDM-1 in 1 *K. pneumoniae* (ST37) and 1 *E. coli* (ST101) isolate. Additional resistance determinants were detected, including CTX-M-15, SHV-11, TEM-1, CMY-2, CMY-4 and CMY-42. Four patients died, including 2 of 3

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patients with CRE bacteremia. There was no evidence of epidemiologic or molecular relatedness between any 2 cases.

Conclusions—This report documents the appearance of highly resistant Gram-negative pathogens in a vulnerable patient population at a pediatric tertiary referral center in a major US metropolitan area. Detailed understanding of the distribution and spread of CRE is essential for the timely detection and containment of these perilous pathogens.

Keywords

carbapenemase; *Klebsiella pneumoniae* carbapenemase; New Delhi metallo-beta-lactamase; multidrug resistance; Gram-negative rods; Los Angeles

Infections due to carbapenem-resistant Enterobacteriaceae (CRE) are on the rise worldwide.[1] In the United States, spread of these highly resistant bacteria has been associated with sporadic occur-rence in pediatric patients in whom few, if any, approved and effective therapeutic options exist.[2–5] Carbapenem resistance results from a variety of molecular mechanisms, either singly or in combination, including carriage of plasmid-borne or chromosomal genes encoding serine carbapenemase or metallo-β-lactamase enzymes; expression of efflux pumps or acquisition of an outer membrane porin mutation in combination with overproduction of Ambler class A (extended-spectrum beta-lactamase-type) or class C (AmpC-type) cephalosporinases.[6] A potential exists for widespread transmission of plasmid-borne carbapenem resistance genes in healthcare and community settings.

Risk factors, resistance characteristics and clinical outcomes of CRE infections in pediatric patients are not well described. Herein, we present the clinical and molecular details of the largest case series to date in a free-standing pediatric hospital in the United States, including 5 cases of carbapenemase-producing Enterobacteriaceae seen between 2011 and 2013 at the Children's Hospital Los Angeles (CHLA).

MATERIALS AND METHODS

Patients and Definition of CRE

CHLA is a 347-bed, free-standing pediatric tertiary care center located in Los Angeles, California. Although no adult care facilities are located on this campus, CHLA does serve a subset of patients over 18 years of age with pediatric issues who have not yet graduated to an adult facility. CHLA does not have a routine screening program for patients with history or travel to a CRE endemic region. There have been no identified CRE outbreaks at this center. In 2011, we began collecting CRE isolates, defined as any Enterobacteriaceae isolate that was non-susceptible to meropenem or imipenem (minimum inhibitory concentration (MIC) 2 μg/mL) and resistant to ceftriaxone (MIC 4 μg/mL), cefotaxime (MIC 4 μg/mL) and ceftazidime (MIC 16 μg/mL), according to contemporary Clinical and Laboratory Standards Institute guidelines (CLSI M100-S23). All isolates were screened for the production of carbapenemase by the modified Hodge test (MHT). Then, to detect any additional CRE retrospectively, we used the breakpoint criteria to query the microbiology laboratory database, which captures antimicrobial susceptibility testing results at our center

dating back to 2005. For all CRE cases, clinical data, including medication history and details of the hospital course, were extracted from the medical record. The CHLA Institutional Review Board approved this study.

Antimicrobial Susceptibility Testing

Specimen processing, bacterial species identification and antimicrobial susceptibility testing were performed by the CHLA Clinical Microbiology Laboratory according to contemporary Clinical and Laboratory Standards Institute standards (M100-S23). MIC values were determined using the Gram-negative susceptibility panel of the Vitek II platform (bioMérieux, Durham, NC), Etest (bioMérieux) and/or the Trek Sensititre broth microdilution panel for Gram-negative bacteria (Trek Diagnostic Systems, Cleveland, OH). To characterize susceptibility to colistin and tigecycline, we used the European Committee on Antimicrobial Susceptibility Testing breakpoint criteria of 2 µg/mL and 1 µg/mL, respectively.

Resistance Genotyping

Genotypic analysis, including polymerase chain reaction (PCR) screening for and sequencing of genes encoding plasmid-encoded enzymes of the carbapenemase (bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{OXA-48}), AmpC (bla_{CMY}) and extended-spectrum beta-lactamase (bla_{CTX-M} and bla_{SHV}) types, as well as characterization of the bla_{KPC}-associated Tn4401 platform, was performed at the Seattle Children's Research Institute. Briefly, PCR reactions were carried out in a total volume of 20 µL reaction mix containing bacterial lysis DNA template, JumpStart Taq polymerase Ready Mix (Sigma-Aldrich Inc., St. Louis, MO) and primers as listed in Table, Supplemental Digital Content 1, http://links.lww.com/INF/B971. PCR cycling conditions have been optimized at different annealing temperatures (T_{ann}) for different primer sets (Table, Supplemental Digital Content 1, http://links.lww.com/INF/B971). PCR reactions were carried out using a C1000 Thermal Cycler (Bio-Rad, Hercules, CA) under the following conditions: initial denaturation (94°C for 2 minutes), followed by 30 cycles of denaturation (94°C for 30 seconds), annealing (at specified T_{ann} per Table, Supplemental Digital Content 1, http://links.lww.com/INF/B971, for 15 seconds) and extension (72°C for 1 minute). PCR-based replicon typing was performed for IncF-related plasmid backbones using the method described by Villa and colleagues,[7] and alleles were assigned using the international plasmid multilocus sequence typing (MLST) database (http://pubmlst.org/plasmid/).

Molecular Epidemiology

MLST was performed at Seattle Children's Research Institute for *Escherichia coli* and *Klebsiella pneumoniae* isolates as previously described.[8,9] Sequence type (ST) assignments were obtained using the Achtman *E. coli* MLST database (http://mlst.warwick.ac.uk/mlst) and the Institut Pasteur *K. pneumoniae* MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/), as appropriate. Then, genetic relatedness was evaluated for all isolates using standard pulsed-field gel electrophoresis (PFGE) by *Xba*I digestion.[10]

Statistical Analysis

Statistical comparison was performed using STATA 10.0 (StataCorp LP, College Station, TX). Wilcoxon-rank sum test was used to compare the number of antibiotic days between groups. A 2-sided P value <0.05 was considered statistically significant.

RESULTS

Clinical Characteristics

Eleven CRE isolates were recovered from 10 patients between April 2011 and May 2013. All patients except 1 (patient 8) had underlying medical conditions (Table 1). Eight patients had indwelling devices, including central lines (n = 8), endotracheal tubes (n = 3) or surgical drains (n = 2); the remaining 2 patients underwent intermittent urinary catheterization multiple times daily. International travel in the year preceding CRE recovery was documented in 2 patients. Eight patients had a documented hospitalization or Emergency Department visit at an outside adult hospital in the year prior to CRE isolation. Patients received a median of 28 antimicrobial days, including 20.5 days on Gram-negative-directed antimicrobials, during the 6 months prior to CRE isolation. Antimicrobial exposure was greater in patients with carbapenemase-producing CRE than in those with carbapenemase-negative CRE, with regard to both Gram-negative-directed antimicrobials (median, 53 vs. 14 days, $P = 0.028$) and carbapenem agents (median, 14 vs. 0 days, $P = 0.007$).

Treatment regimens and duration varied for each of the 7 episodes of CRE infection in 6 patients (Table 1). In 4 patients, the CRE isolate was not considered to be a pathogen; thus, antimicrobial treatment was not started. Six patients were hospitalized for a mean of 39.3 days (range: 5–74 days). Four patients died during the hospitalization in which CRE was recovered, including 2 of 3 (66.7%) with CRE bacteremia.

Antimicrobial Susceptibility Testing

Susceptibility to typical and alternative non-beta-lactam agents varied between organisms (Table 1), although all 11 isolates appeared susceptible to colistin and tigecycline.

Carbapenemase and Cephalosporinase Resistance Genotypes

Carbapenemase genes were identified in 5 isolates. Three isolates from 3 patients encoded Ambler class A KPC-3 carbapenemases, including 2 *K. pneumoniae* and 1 *E. coli*. Two of the bla_{KPC-3} genes (1 each in *K. pneumoniae* and *E. coli*) appeared in the genetic context of the Tn*4401*b transposon isotype with no *bla* promoter deletion, while the remaining bla_{KPC-3} appeared in the Tn*4401*d isotype, featuring a 68-bp deletion. Three isolates from 2 patients carried Ambler class B NDM-1 carbapenemase, including an *E. coli* in a patient who had received medical care in India, and a *K. pneumoniae* that was isolated initially from urine and then from blood in a patient with no known travel history. All *E. coli* and all but 1 *K. pneumoniae* isolates also encoded extended-spectrum cephalosporinase enzymes from Ambler classes A (CTX-M) and C (CMY; Table, Supplemental Digital Content 1, http://links.lww.com/INF/B971).

Genetic Relatedness

MLST and PFGE were used to characterize genetic relatedness among the 11 CRE isolates (Fig. 1). MLST profiles and PFGE fingerprints of the 2 isolates from patient 3 were indistinguishable, as expected. There was no evidence of strain relatedness between any 2 patients.

Case Summaries

The 5 patients with carbapenemase-producing isolates are described here.

Case 1—A 20-year-old male who suffered an abdominal gunshot wound at age 16 years, resulting in multiple bowel resections, complained of abdominal pain at a routine gastroenterology clinic visit but was otherwise in stable condition. Gastrostomy tube secretions were cultured and grew *K. pneumoniae* resistant to carbapenems. The culture was considered to represent colonization rather than infection, and the patient was not admitted for treatment.

In the 12 months prior to CRE recovery, he had received multiple courses of antimicrobials for central line-associated and abdominal wound infections. Of note, 9 months prior to CRE recovery, he had central line-associated sepsis with highly susceptible *K. pneumoniae* (resistant only to ampicillin) that was treated with piperacillin–tazobactam for 14 days. Six months prior to CRE recovery, he was treated with 17 days of meropenem for a wound infection due to extended-spectrum cephalosporin-resistant *K. pneumoniae*. The patient did have a Broviac central line and gastrostomy tube in place at the time of CRE recovery. He had visited emergency departments in adult hospitals but had no admissions to hospitals with known CRE outbreaks. He had not traveled outside of the United States.

Case 2—A 7-month-old infant with hemophagocytic lymphocytosis diagnosed at 5 months of age was transferred to CHLA from an outside hospital for bone marrow transplantation evaluation. She had traveled to Lebanon with family prior to her diagnosis. She had received etoposide and high-dose steroids. Due to high fever on admission, she was started on meropenem. Fever initially resolved but recurred on meropenem, accompanied by emesis and diarrhea; stool tested positive for toxigenic *Clostridium difficile,* and oral vancomycin therapy was initiated. Blood culture drawn on hospitalization day (HD) 17 grew *K. pneumoniae*; amikacin was added. Based on a positive MHT and further susceptibility testing, colistin was also added. The Broviac line was removed on HD 23, but blood cultures remained persistently positive for *K. pneumoniae* on HD 23, 24, 26 and 27. Ertapenem was added on HD 27.[11] After the patient developed pneumatosis, Allow Natural Death orders were put in place, on HD 27; no further blood cultures were obtained. The patient died on HD 32.

Case 3—A 3-year-old male with gangliosidosis and tracheostomy/ ventilator dependence, who had been previously treated at another Los Angeles hospital, was admitted to CHLA for his 5th cardiopulmonary arrest, ultimately attributed to respiratory infection with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. He remained hospitalized for management of diabetes insipidus. Following multiple daily urinalysis samples with positive

leukocyte esterase results, a urine specimen obtained on HD 31 grew >100,000 CFU/ mL of *P. aeruginosa* and 50–100,000 CFU/mL carbapenem-resistant *K. pneumoniae* (CRKP). Repeat urine culture on HD 32 showed pure growth of >100,000 CFU/mL CRKP. He received a 14-day course of ciprofloxacin via gastrostomy tube; follow-up urine culture was negative. On HD 51, he developed fever, tachycardia and increased work of breathing. Blood culture from the peripherally inserted central catheter (PICC) grew *K. pneumoniae* with MICs of 4, 2 and 1 for meropenem, doripenem and imipenem, respectively. He was thus started on imipenem and colistin (MIC 0.25). Blood cultures remained positive until the PICC was pulled on HD 56. He received 21 additional days of antimicrobial therapy.

Case 4—A 23-year-old female with Blue Rubber Bleb Nevus syndrome, paraplegia, and neurogenic bowel and bladder requiring intermittent catheterization who received weekly blood transfusions at CHLA presented to a scheduled appointment with headache and hypertension. She was noted to have amber-colored urine with a foul smell. The urine culture grew carbapenem-resistant *E. coli*. She completed a 10-day course of nitrofurantoin. However, symptoms recurred 15 days later and a repeat urine culture was positive for the same organism. She completed a second 10-day course of nitrofurantoin with symptomatic improvement.

Although case 4 was of adult age, the patient continued to receive her outpatient care by pediatric specialists at our facility. She had 1 inpatient admission to an outside community hospital during the 6 months prior to CRE isolation.

Case 5—A 2-year-old girl with myelodysplastic syndrome remarkable for abnormal bone marrow cytogenetics (monosomy 7 and trisomy 8) and recurrent fever was transferred to CHLA from India for bone marrow transplantation evaluation 7 months prior to CRE recovery. Two months prior to CRE isolation, the patient was admitted with fever, neutropenia, abdominal distention and bloody diarrhea. Blood culture obtained on HD 13 grew *K. pneumoniae* resistant only to ampicillin; she was treated with cefotaxime. When blood cultures drawn on HD 19 and 20 grew *K. pneumoniae* and *E. coli* resistant to third-generation cephalosporins but susceptible to meropenem (MIC 0.094 and 0.023, respectively) and ampicillin-resistant *Enterococcus faecium*, therapy was switched to meropenem and vancomycin and given for 14 days. The PICC was removed on HD 20, and follow-up blood cultures were negative. A new PICC was placed on HD 32. On HD 45, she developed sepsis due to *K. pneumoniae* resistant to third-generation cephalosporins but again susceptible to meropenem (MIC 0.064). She was started on meropenem, with negative follow-up cultures. The central line was not removed.

On HD 75, on treatment day 30 of meropenem, she developed bacteremia with carbapenem-resistant *E. coli*. Meropenem was discontinued and colistin, amikacin and imipenem were added on HD 76, 79 and 80, respectively. Liposomal amphotericin was started on HD 76. On HD 80, a new black lesion was found on the hard palate. Computed tomography scan showed extensive bilateral maxillary and ethmoid sinusitis. The lesion was biopsied immediately and grew *Aspergillus flavus*. Voriconazole was added to liposomal amphotericin. Allow Natural Death orders were instituted. The patient died on HD 89.

DISCUSSION

This is the largest case series of pediatric CRE at a free-standing children's hospital in the United States. KPC-positive *K. pneumoniae* were first reported in adult patients in 2002 in New York state. They spread rapidly throughout the Northeastern United States,[12] and by 2008, were prevalent in the Midwest as well.[13,14] The Western United States remained largely unaffected until fall 2009, when the Centers for Disease Control and Prevention (CDC) contacted the Los Angeles County Department of Public Health (LACDPH) regarding detection of identical CRKP in adult patients at several local hospitals. LACDPH declared CRKP a laboratory-reportable disease in June 2010, with 814 cases being reported in the next 12 months.[15] As of February 2014, KPC had been detected in at least 47 states (www.cdc.gov/hai/organisms/cre/TrackingCRE.html), with some 70% of KPC-producing *K. pneumoniae* received by the CDC belonging to a single strain, *K. pneumoniae* ST258.[16] The KPC-3-positive *K. pneumoniae* in our pediatric institution were detected in April 2011 (ST18) and February 2012 (ST258), at least 1 year after the initial detection of CRKP in Los Angeles County; the distinctive Tn*4401* isotype profiles associated with bla_{KPC} in these 2 isolates suggested carriage of distinctive plasmids.

KPC has been detected in other Enterobacteriaceae species as well, including *Klebsiella oxytoca* and *Enterobacter* spp.[17–19] More concerning, however, has been the detection of KPC in the globally disseminated and highly virulent, multidrug-resistant *E. coli* ST131 clone.[20] Kim and colleagues described the recovery in Pittsburgh of 7 KPC-2 and KPC-3-positive *E. coli* ST131 from September 2008 to February 2011, including 4 with identical plasmid restriction patterns, indicating clonal spread.[21] The KPC-3-positive *E. coli* ST131 in our study (case 4) was detected in November 2011, but the extent of its relatedness to strains from the earlier East Coast cluster remains unclear. Likewise, though this bla_{KPC-3} was associated with the Tn*4401*b isotype, we are unable to comment without further study on the possibility of plasmid sharing between this *E. coli* and the *K. pneumoniae* isolate (case 1) exhibiting this molecular profile.

In 2010, infections due to *E. coli* and *K. pneumoniae* carrying the New Delhi Metallo-beta-lactamase (NDM) carbapenemase were first reported in patients returning to Western Europe from medical tourism travels in India and Pakistan.[22] NDM subsequently spread throughout Europe, driven in large part by population mobility, including recreational and medical travel.[23] In June 2010, the CDC reported the first cases of NDM-positive Enterobacteriaceae in the United States,[24] with the first of these having been recovered in April 2009. NDM-producing *K. pneumoniae* was first recovered in California in December 2009, with 3 additional cases recovered from September 2010 to March 2011, including 1 pediatric patient in Los Angeles County[2]; all 4 cases occurred in patients that had been hospitalized in India or Pakistan.[25] Carriage of NDM-1 plasmids in the *E. coli* ST101 strain background has been well documented in isolates from India,[26,27] consistent with our patient's history of birth and medical care in India. However, while NDM-1 plasmids have also been reported in *K. pneumoniae* ST37 isolates from India and the UK,[28] our patient with NDM-positive *K. pneumoniae* had no documented history of international travel. Detection of NDM positivity in a strain recovered from a patient without known history of travel abroad should raise concern for the possibility of person-to-person spread of

established NDM-associated plasmids or clones in the community or within the healthcare network.

Carbapenemase-positive *Enterobacter* spp. have been described in North America,[18] but no plasmid-borne carbapenemases (or cephalosporinases) were detected in the *Enterobacter* isolates in this study. The positive MHT results in these isolates suggest the production of beta-lactam-hydrolyzing enzymes, such as de-repressed chromosomal AmpC determinants or constitutively expressed plasmid-borne enzymes that were not captured by our panel. Production of such enzymes in combination with an outer membrane porin deficiency can produce a clinically relevant phenotype of carbapenem resistance[29] as well as a false-positive MHT result.[30]

We included 1 adult age patient (case 4) who received ongoing care at our pediatric facility. Care of patients over 18 years of age is not unusual at an academic referral center, as care has improved for diseases that were once considered strictly pediatric conditions. These patients may have the additional risk of receiving care in adult facilities where CRE are more prevalent.

CRE infection has been associated with high mortality rates in adults.[31–33] The Emerging Infections Network recently published a case series describing the difficulties in management of 85 source patients with CRE reported from across the United States.[34] CRE was the direct cause or exacerbated conditions leading to death in half of our 6 patients with CRE infection. The 3 fatal cases represented the most vulnerable segments of the pediatric population, including neonates and those with hematologic malignancies, who require complex care and routinely receive empiric antibiotic courses. Prolonged and repeated broad-spectrum antibiotic exposure is detrimental to the richly diverse intestinal flora that provide colonization resistance against overgrowth of typically minor constituents of the flora such as Enterobacteriaceae.[35] Although the treatment of CRE infection is confounded by the fact that patients who tend to receive broad-spectrum antibiotics are also those at greatest risk of complications from infection (and at risk of dose-limiting toxicity from combination antibiotics), the principles and practices of Antimicrobial Stewardship[36] are critical to preserve the dwindling therapeutic options that remain for treatment of these highly resistant pathogens.

Even in a metropolitan area with high-CRE prevalence, pediatric infection with these perilous pathogens remains rare. The cases we describe in this report appear to represent sporadic cases with no molecular or epidemiologic links to one another. Although 2 cases of CRE had clear history of international travel and molecular features associated with international clones, 1 case of NDM-positive *K. pneumoniae* infection did not, raising the alarming possibility of community or healthcare network spread of the associated global clone. LACDPH surveillance has identified CRKP circulating throughout the healthcare community as a whole and especially within long-term acute care hospitals.[15] The spread of CRE infections into pediatric populations highlights the need for active partnerships between healthcare facilities and their local public health jurisdiction, which may lead to better designed interventions to interrupt CRE transmission within and between health-care

facilities. It also magnifies the need for robust Antimicrobial Stewardship programs to reduce vulnerability to colonization and infection with these organisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

REFERENCES

1. Centers for Disease Control and Prevention. Vital signs: carbapenem-resistant Enterobacteriaceae. MMWR Morb Mortal Wkly Rep. 2013; 62:165–170. [PubMed: 23466435]

2. Mochon AB, Garner OB, Hindler JA, et al. New Delhi metallo-β-lactamase (NDM-1)-producing Klebsiella pneumoniae: case report and laboratory detection strategies. J Clin Microbiol. 2011; 49:1667–1670. [PubMed: 21325558]

3. Logan LK. Carbapenem-resistant enterobacteriaceae: an emerging problem in children. Clin Infect Dis. 2012; 55:852–859. [PubMed: 22700827]

4. Little ML, Qin X, Zerr DM, Weissman SJ. Molecular diversity in mechanisms of carbapenem resistance in paediatric Enterobacteriaceae. Int J Antimicrob Agents. 2012; 39:52–57. [PubMed: 22055532]

5. Dara JS, Chen L, Levi MH, Kreiswirth BN, Pellett Madan R. Microbiological and genetic characterization of carbapenem-resistant Klebsiella pneumoniae isolated from pediatric patients. J Pediatric Infect Dis Soc. 2014; 3:e10–e14. [PubMed: 24567846]

6. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clin Infect Dis. 2011; 53:60–67. [PubMed: 21653305]

7. Villa L, García-Fernández A, Fortini D, Carattoli A. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother. 2010; 65:2518–2529. [PubMed: 20935300]

8. Wirth T, Falush D, Lan R, et al. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol. 2006; 60:1136–1151. [PubMed: 16689791]

9. Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. Multilocus sequence typing of Klebsiella pneumoniae nosocomial isolates. J Clin Microbiol. 2005; 43:4178–4182. [PubMed: 16081970]

10. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995; 33:2233–2239. [PubMed: 7494007]

11. Bulik CC, Nicolau DP. Double-carbapenem therapy for carbapenemase-producing Klebsiella pneumoniae. Antimicrob Agents Chemother. 2011; 55:3002–3004. [PubMed: 21422205]

12. Bratu S, Landman D, Haag R, et al. Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City: a new threat to our antibiotic armamentarium. Arch Intern Med. 2005; 165:1430–1435. [PubMed: 15983294]

13. Marchaim D, Chopra T, Pogue JM, et al. Outbreak of colistin-resistant, carbapenem-resistant Klebsiella pneumoniae in metropolitan Detroit, Michigan. Antimicrob Agents Chemother. 2011; 55:593–599. [PubMed: 21115786]

14. Marchaim D, Chopra T, Perez F, et al. Outcomes and genetic relatedness of carbapenem-resistant Enterobacteriaceae at Detroit medical center. Infect Control Hosp Epidemiol. 2011; 32:861–871. [PubMed: 21828966]

15. Marquez P, Terashita D, Dassey D, et al. Population-based incidence of carbapenem-resistant Klebsiella pneumoniae along the continuum of care, Los Angeles County. Infect Control Hosp Epidemiol. 2013; 34:144–150. [PubMed: 23295560]

16. Kallen AJ, Srinivasan A. Current epidemiology of multidrug-resistant gram-negative bacilli in the United States. Infect Control Hosp Epidemiol. 2010; 31(Suppl 1):S51–S54. [PubMed: 20929371]

17. Mathers AJ, Cox HL, Kitchel B, et al. Molecular dissection of an outbreak of carbapenem-resistant Enterobacteriaceae reveals intergenus KPC carbapenemase transmission through a promiscuous plasmid. MBio. 2011; 2:e00204–e00211. [PubMed: 22045989]

18. Haraoui LP, Lévesque S, Lefebvre B, et al. Polyclonal outbreak of KPC-3-producing Enterobacter cloacae at a single hospital in Montreal, Quebec, Canada. J Clin Microbiol. 2013; 51:2406–2408. [PubMed: 23637289]

19. Marchaim D, Navon-Venezia S, Schwaber MJ, et al. Isolation of imipenem-resistant Enterobacter species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. Antimicrob Agents Chemother. 2008; 52:1413–1418. [PubMed: 18227191]

20. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, et al. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008; 61:273–281. [PubMed: 18077311]

21. Kim YA, Qureshi ZA, Adams-Haduch JM, Park YS, Shutt KA, Doi Y. Features of infections due to Klebsiella pneumoniae carbapenemase-producing Escherichia coli: emergence of sequence type 131. Clin Infect Dis. 2012; 55:224–231. [PubMed: 22491340]

22. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010; 10:597–602. [PubMed: 20705517]

23. Cantón R, Akóva M, Carmeli Y, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect. 2012; 18:413–431. [PubMed: 22507109]

24. Centers for Disease Control and Prevention. Detection of Enterobacteriaceae isolates carrying metallo-beta-lactamase - United States, 2010. MMWR Morb Mortal Wkly Rep. 2010; 59:750. [PubMed: 20577157]

25. Rasheed JK, Kitchel B, Zhu W, et al. New Delhi metallo-β-lactamase-producing Enterobacteriaceae, United States. Emerg Infect Dis. 2013; 19:870–878. [PubMed: 23731823]

26. Poirel L, Dortet L, Bernabeu S, Nordmann P. Genetic features of blaNDM-1-positive Enterobacteriaceae. Antimicrob Agents Chemother. 2011; 55:5403–5407. [PubMed: 21859933]

27. Mushtaq S, Irfan S, Sarma JB, et al. Phylogenetic diversity of Escherichia coli strains producing NDM-type carbapenemases. J Antimicrob Chemother. 2011; 66:2002–2005. [PubMed: 21669947]

28. Giske CG, Fröding I, Hasan CM, et al. Diverse sequence types of Klebsiella pneumoniae contribute to the dissemination of blaNDM-1 in India, Sweden, and the United Kingdom. Antimicrob Agents Chemother. 2012; 56:2735–2738. [PubMed: 22354295]

29. Fernández L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. Clin Microbiol Rev. 2012; 25:661–681. [PubMed: 23034325]

30. Thomson KS. Extended-spectrum-beta-lactamase, AmpC, and carbapenemase issues. J Clin Microbiol. 2010; 48:1019–1025. [PubMed: 20181902]

31. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant Klebsiella pneumoniae infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol. 2008; 29:1099–1106. [PubMed: 18973455]

32. Gasink LB, Edelstein PH, Lautenbach E, Synnestvedt M, Fishman NO. Risk factors and clinical impact of Klebsiella pneumoniae carbapenemase-producing K. pneumoniae. Infect Control Hosp Epidemiol. 2009; 30:1180–1185. [PubMed: 19860564]

33. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant Klebsiella pneumoniae acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother. 2008; 52:1028–1033. [PubMed: 18086836]

34. Drekonja DM, Beekmann SE, Elliott S, et al. Challenges in the management of infections due to carbapenem-resistant Enterobacteriaceae. Infect Control Hosp Epidemiol.

35. Vollaard EJ, Clasener HA. Colonization resistance. Antimicrob Agents Chemother. 1994; 38:409–414. [PubMed: 8203832]

36. Dellit TH, Owens RC, McGowan JE, et al. Infectious diseases society of America and the society for healthcare epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. Clin Infect Dis. 2007; 44:159–177. [PubMed: 17173212]

37. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011; 17:1791–1798. [PubMed: 22000347]

38. Batchelor M, Hopkins K, Threlfall EJ, et al. bla(CTX-M) genes in clinical Salmonella isolates recovered from humans in England and Wales from 1992 to 2003. Antimicrob Agents Chemother. 2005; 49:1319–1322. [PubMed: 15793104]

39. Yan JJ, Wu SM, Tsai SH, et al. Prevalence of SHV-12 among clinical isolates of Klebsiella pneumoniae producing extended-spectrum beta-lactamases and identification of a novel AmpC enzyme (CMY-8) in Southern Taiwan. Antimicrob Agents Chemother. 2000; 44:1438–1442. [PubMed: 10817689]

40. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002; 40:2153–2162. [PubMed: 12037080]

41. Barroso H, Freitas-Vieira A, Lito LM, et al. Survey of Klebsiella pneumoniae producing extended-spectrum beta-lactamases at a Portuguese hospital: TEM-10 as the endemic enzyme. J Antimicrob Chemother. 2000; 45:611–616. [PubMed: 10797082]

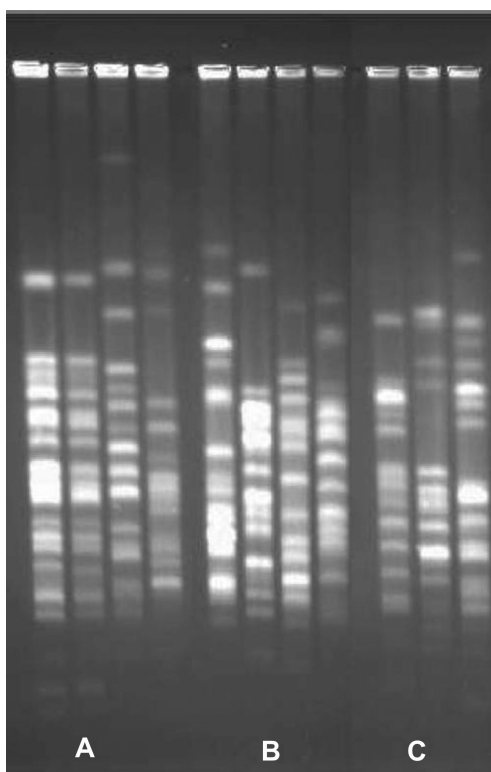**FIGURE 1.**
PFGE fingerprints of 11 carbapenem-resistant Enterobacteriaceae. Panel A: *K. pneumoniae* cases #3 (1st and 2nd episodes), #1 and #2. Panel B: *E. coli* cases #7, #6, #5 and #4. Panel C: *E. cloacae* cases #9, #10 and #8.

TABLE 1

Clinical, microbiologic and molecular characteristics of 10 pediatric patients colonized or infected with CRE

#	Date	Clinical Features	Risk Factors						Microbiology/Molecular Features						Hospital Course			
			Travel	OSH	Antibiotic Days prior to CRE Isolation†			Site	AST Results	Carba-penemase	Other Genes	MLST	IncF plasmid type	Treatment	ICU	Hosp days	Death
					All	GN	CP										
		Klebsiella pneumonia															
1	2011	20 y hx abd GSW p/w abd pain	-	Y	112	87	13	W	-	KPC-3 (Tn4401b)	-	ST18	K4	None	N	0	N
2	2012	7 m hx HLH p/w sepsis	LEB	Y	26	20	15	B	CIP, GM, T/S	KPC-3 (Tn4401d)	-	ST258	-	ERT, MER, COL	N	17	Y
3*	2013 then 2013	3 y hx gangliosidosis p/w UTI, then sepsis	-	Y	57	53	2	U, then B	CIP, GM, T/S	NDM-1	CTX-M-15, CMY-4, SHV-11	ST37	-	LEV then IMI, COL	Y	55	N
		Escherichia coli															
4	2011	24 y hx blue rubber bleb nevus syndrome and neurogenic bladder p/w UTI	-	Y	21	21	21	U	CIP, GM, T/S	KPC-3 (Tn4401b)	CTX-M-15, TEM-1	ST131	F-:A2:B20	NIT	N	0	N
5	2012	2 y hx MDS p/w sepsis	IND	Y	158	111	93	B	CIP, GM, T/S	NDM-1	CTX-M-15, CMY-42	ST101	F2:A1:B20	IMI, AMI	N	74	Y
6	2011	16 y hx spina bifida p/w UTI	-	N	14	14	0	U	CIP, GM, T/S	-	CTX-M-15	ST10	F31:A4:B1	MER	N	0	N
7	2012	11 y dx lipomeningocele, neurogenic bladder	-	N	0	0	0	U	-	-	CMY-2, TEM-1	ST457	-	None	N	0	N
		Enterobacter cloacae															
8	2012	5 y s/p drowning, p/w respiratory failure	-	Y	4	4	0	R	-	-	-	-	-	None	Y	5	Y
9	2012	3 m neuroblastoma p/w increased resp secretions	-	Y	30	16	0	R	-	-	-	-	-	None	Y	23	N
10	2012	1 m ex-34 week premature infant, trisomy 21, NEC p/w abd abscess	-	Y	70	39	0	W	-	-	-	-	-	FEP, TOB	Y	44	Y

AMI indicates amikacin; AST, antibiotic susceptibility testing (phenotypic resistance); B, blood; CIP, ciprofloxacin; CP, carbapenem; FEP, cefepime; GSW, gunshot wound; GM, gentamicin; GN, Gram-negative spectrum (including 2nd-, 3rd- or 4th-generation cephalosporins, beta-lactam/beta-lactamase inhibitor combinations, carbapenems, aminoglycosides, or fluoroquinolones); HLH, hemophagocytic lymphohistiocytosis; Hosp, hospitalization; hx, history of; IND, India; LEB, Lebanon; m, month; MDS, myelodysplastic syndrome; MER,

meropenem; MLST, multiple locus sequence typing; N, no; NEC, necrotizing enterocolitis; OSH, outside hospital care; p/w, presented with; R, respiratory tract; ST, sequence type; TOB, tobramycin; T/S, trimethoprim/sulfamethoxazole; U, urine; UTI, urinary tract infection; W, wound; y, year; Y, yes.

*
CRE was isolated from urine and blood 19 days apart in patient 3 as described in the text.

†
Antimicrobial exposure days during the 6 months prior to CRE isolation.